Immunity
Previews

Islet β-Cell Death—Fuel to Sustain Autoimmunity?

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DOI 10.1016/j.immuni.2007.08.002

Under what context do dying β-cells enhance the autoimmune process in type 1 diabetes? Kim et al. (2007) find that secondary necrosis of β-cells can prime the autoimmune response via uptake by and activation of antigen-presenting cells through Toll-like receptor 2.

The notion that it indeed "matters how you die" has been investigated by numerous research groups interested in autoimmune disorders. Cell death may result in either tolerance or enhanced autoimmunity, and in type 1 diabetes (T1D), evidence has been found for both scenarios. The prevailing thought is that antigens from cells under attack can provide new fuel for the autoimmune response when they are taken up by professional antigen-presenting cells (APCs) in the pancreatic lymph node, where antigen spreading as well as targeting of neoantigens occurs. Conversely, apoptotic β-cell death can induce tolerance and switch autoreactive T cells off. These findings lead to the hypothesis that "good" apoptotic but not "bad" necrotic cell death is required for maintaining peace in the body (Gallucci et al., 1999). However, it has become increasingly clear that a simple distinction between good and bad cell death is difficult to establish and that the precise degree and context of cellular demise determines whether an autoimmune process is sustained or switched off.

In this issue of *Immunity*, Kim et al. (2007) find that apoptotic cells undergoing secondary necrosis, but not intact apoptotic cells, could induce tumor necrosis factor (TNF) production by macrophages and activation of autoreactive T cells similar to primary necrotic cells. In addition, secondary necrotic cells appear to trigger T cell immunity through a Toll-like receptor 2 (TLR2)-MyD88-NF-κB signaling pathway. Importantly, autoimmune diabetes is markedly inhibited in TLR2-deficient mice as a consequence of impaired priming of diabetogenic T cells by APCs after β-cell injury. The results presented here thus suggest that TLR2-dependent stimulation of APCs by secondary necrotic apoptotic β-cells may trigger and sustain the autoaggressive immune response in autoimmune diabetes. How can we reconcile the present findings with previous data implicating TLR2 signaling in enhancing immune regulation (Zanin-Zhorov et al., 2006) and, more importantly, with a previous report (Hugues et al., 2002) demonstrating that a limited degree of β-cell apoptosis can decrease the incidence of type 1 diabetes in nonobese diabetic (NOD) mice?

To better understand the dilemma of whether target cell death will fuel or quench the autoimmune response, it is important to consider several factors: First is the degree of apoptosis and manner of cell death. On the basis of the present report and previous evidence, the emerging theme is that secondary necrotic cell death renders induction of autoimmune immune responses more likely because APCs are more efficiently primed, possibly by recognizing damage-associated molecular patterns (DAMPs) (Gallucci et al., 1999). In addition, it appears that only limited apoptosis has to occur in order for tolerance to be achieved (Hugues et al., 2002).

The second factor is the precise timing of cell death in relation to the stage of the autoimmune response. Hugues et al. (2002) gave 40 mg/kg streptozotocin to 4-week-old NOD mice and prevented T1D in greater than 50% of the animals. In contrast, when administering the same drug to older NOD mice, Kim et al. (2007) found TLR2-dependent acceleration of the disease. On the basis of these findings, it is reasonable to hypothesize that the precise degree of pre-existing inflammation and islet destruction will determine the likelihood for apoptotic β-cells to induce tolerance. In this context, the presence of other inflammatory factors such as TNF, interferons, and possibly certain TLR ligands should be considered.

Third, the precise balance between regulatory (Treg) and effector T cells will probably determine which cell population is more efficiently primed by APCs that engulf and present autoantigens. It is clear that the state of the APCs is a major factor determining whether Treg or effector T cells are primed or whether their anergy or deletion occurs. In this respect, many inflammatory mediators are also required for maintaining regulatory cells and may constitute important factors for curbing the effector response through apoptosis (Figure 1 and Discussion below). Thus, the number and location of effector T cells versus Treg cells reactive to islet antigens, as well as their likelihood to encounter APCs that are in a tolergoneic or activating state, will in the end determine whether prevention or enhancement of autoimmunity occurs.

Interestingly, in recent reports it has become clear that inflammatory factors such as TNF, interferons, or TLR2 ligands can paradoxically reduce inflammation through the selective induction of Treg cells or by enhancing apoptosis of effector cells. Conversely, certain factors with known regulatory function such as TGF-β have dual effects and can in some cases enhance

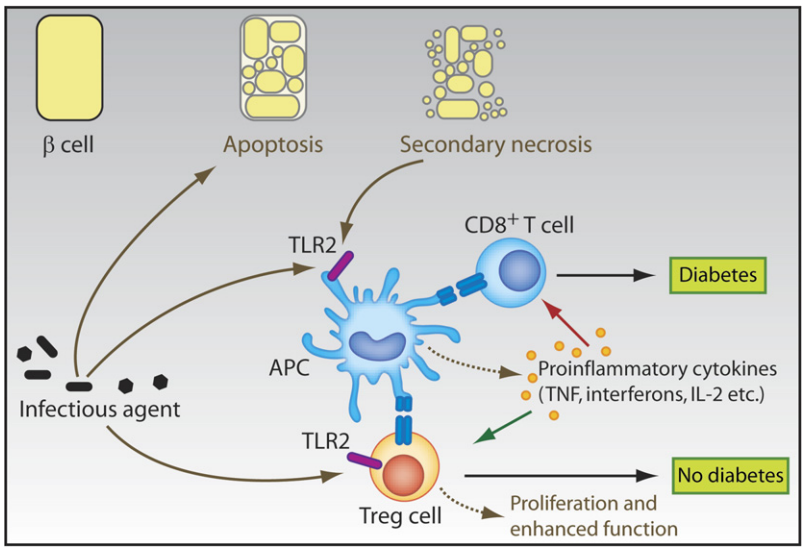

Figure 1. Induction or Abrogation of Type 1 Diabetes under Inflammatory Conditions
Kim et al. (2007) observe that secondary necrosis of β-cells can prime the autoimmune response in type 1 diabetes by activating APCs through TLR2. Ensuing TNF production by these cells induces the activation of autoreactive T cells that cause diabetes. Current understanding suggests a dual role of TLR2 and proinflammatory cytokines such as TNF in autoimmunity. Engagement of TLR2 on Treg cells or APCs, for example during a microbial infection (which may in some cases also cause β-cell death), can induce the proliferation of Treg cells and enhance their capacity to control pathogenic effector T cells. Similarly, TNF, and possibly other proinflammatory cytokines, can have a beneficial role in autoimmune diabetes by stimulating Treg cell expansion and function or by inducing apoptosis of autoreactive T cells.

inflammation. Thus, several reports have shown that TLR2 might be crucial for the activation and maintenance of Treg cells. This is evidenced not only in the mouse from studies involving TLR2-deficient animals or TLR2 agonists in vitro (Sutmuller et al., 2006) but also in human type 1 diabetes from studies involving hsp277, a heat-shock-protein-derived peptide that induces tolerance in this disease (Zanin-Zhorov et al., 2006). All these studies point to a role for TLR2 in its capacity to enhance Treg cells proliferation, which can occur even under inflammatory conditions. We do not understand the difference in function compared with the present report but can hypothesize that the timing and degree of TLR2 engagement may be crucial. In addition, presence in the microenvironment of particular APC types and inflammatory mediators, for example as a consequence of microbial infection, will probably have crucial influence on the outcome of TLR ligation in autoimmunity.

TNF is an interesting molecule that can be important for controlling (Wu et al., 2002) as well as priming Treg cells (Chen et al., 2007). TNF can enhance inflammation and autoimmune diabetes when present early during diabetogenesis, but it also switches off effector cells and prevents diabetes by inducing apoptosis when it is present at later stages (Christen et al., 2001). On the basis of these observations, it might be extremely difficult, if not impossible, to alter TNF signaling pathways in type 1 diabetes therapeutically. Yet, it is of interest to mention here that multifaceted effects can be strictly disease dependent. Thus, in some cases, for example in arthritis where TNF predominantly enhances inflammation and curbs Treg cell activity, this cytokine is suitable as a therapeutic target.

Last, it is noteworthy to point out that TGF-β, which has been portrayed as a hallmark regulatory cytokine because of its role in the development and function of Foxp3$^+$ Treg cells, is now known to exhibit dual functions as well. Its effects are context dependent, with IL-6 conferring TGF-β a crucial role in differentiating IL-17-producing cells that can have destructive effects in autoimmunity (Bettelli et al., 2006).

For type 1 diabetes and probably other autoimmune disorders, our challenge in the future will be to precisely model and understand the balance of effector and regulatory cells and their location in context with the development of β-cell destruction. As discussed here, most factors such as TNF, interferons, and certain TLR ligands can have positive as well as negative effects on the evolving autoimmune response depending on how efficiently they activate the existing Treg cell compartment, how many effector T cells they induce, and how much apoptosis of these effector cells ensues. The type, number, and activation state of APCs in the pancreatic lymph node is also important to consider, and the reality might be that at any given time, APCs with tolerogenic versus immunogenic phenotypes are present. A recently developed tool might be of help in guiding our experimentation and better interpreting our findings: the virtual NOD mouse (Zheng et al., 2007). This model can illustrate unexpected yet important aspects of the autoimmune response in T1D, for example the accumulation of Treg cells that are in the islets of mice with advanced diabetes and that appear to be unable to control effector T cells. Ultimately, such modeling will allow immunological profiling on a per-animal basis and more targeted deployment of immune-based interventions that aim at reducing effector T cells or enhancing Treg cells. This will be instrumental for all factors that have been shown to have opposing effects on the autoimmune process depending on the immunological context (β-cell death, TLRs, TNF, interferons, and more recently TGF-β). Until we have reached this degree of insight, we will be well advised to focus interventions on factors that appear to have more uniform effects in tolerizing the immune system. These include IL-10 and IL-4 induction, mucosal antigen administration, and the use of short-term courses of systemic immunosuppressants for paving a way to safe and efficient Treg cell induction.

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The Expanding Universe of Regulatory T Cell Subsets in Cancer

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DOI 10.1016/j.immuni.2007.08.001

Evidence has indicated that failed antitumor immunity is dominated by immunosuppressive mechanisms within the tumor microenvironment. In this issue of *Immunity*, Peng et al. (2007) add to this list by describing tumor-infiltrating γδ T cells that have regulatory function.

Substantial evidence has been accumulated indicating that immunosuppressive mechanisms within the solid-tumor microenvironment provide a major barrier to effective antitumor immunity. One such mechanism appears to be through the accumulation of $CD4^+CD25^+Foxp3^+$ regulatory T (Treg) cells. Treg cells have been shown to be present at increased numbers in both the peripheral blood and among the tumor-infiltrating lymphocyte (TIL) population in both murine models and in patients with any of several histologies of cancer. Mechanistically, murine models of Treg cell depletion (usually with CD25 monoclonal antibody) have demonstrated improved immune-mediated tumor control, arguing that they are functionally important. Their role directly within the tumor microenvironment is supported by experiments in which anti-CD25 was injected directly into the tumor, and this facilitated tumor regression. In addition, two-photon in vivo imaging has demonstrated that Treg cells can suppress the cytolytic activity of activated $CD8^+$ T cells in a target tissue through an indirect mechanism that prevents lysis but not conjugate formation with target cells (Mempel et al., 2006). Both naturally thymically derived Treg cells and peripherally induced Treg cells have been implicated in tumor models, the latter of which is consistent with the high amounts of transforming growth factor β (TGF-β) observed to be produced in the tumor microenvironment. Although there are not yet definitive mechanistic data showing improved clinical response with Treg cell depletion in human cancer patients, one study has shown a greater induction of antitumor effector T cells when Treg cell numbers were reduced by the administration of a CD25-targeted toxin (Dannull et al., 2005).

However, whether $CD4^+CD25^+FoxP3^+$ Treg cells represent the only lymphocyte population involved in suppressing effector T cell function within the tumor microenvironment is not clear. A role for B cells in suppressing T cell-dependent tumor rejection has been identified in several murine model systems (Qin et al., 1998). Interleukin10 (IL-10)-producing $CD8^+$ T cells, and $CD4^-CD8^-$ T cells, also have been identified (Shevach, 2006) that appear to inhibit the activation of conventional $CD8^+$ T cells in the tumor context. In this issue of *Immunity*, Peng et al. (2007) provide convincing evidence that γδ T cell receptor (TCR)-expressing T cells derived from the tumor microenvironment can have potent suppressive activity toward conventional T cells both in vitro and in vivo, an observation that could have important clinical relevance.

γδ T cells have been implicated as participants in multiple facets of antitumor immune responses, predominantly as positive regulators. In murine studies in vivo, γδ T cells were found to be recruited to tumor sites rapidly, appearing before αβ T cells and producing high amounts of interferon-γ (IFN-γ). Deficiency of γδ T cells